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Regulation and function of transaldolase isoenzymes involved in sugar and one-carbon metabolism in the ribulose monophosphate cycle methylotroph *Arthrobacter* P1

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Abstract. In the facultative methylotroph *Arthrobacter* P1 the enzyme transaldolase plays an important role in both the pentose phosphate pathway and in the ribulose monophosphate cycle of formaldehyde fixation.

Among gluconate-negative mutants of *Arthrobacter* P1 strains occurred which also were unable to grow on xylose because they had lost the ability to synthesize transaldolase. Furthermore, this loss of transaldolase activity resulted in decreased growth rates on a number of other "heterotrophic" substrates. Contrary to expectation, these mutants still grew on methylamine and were even able to use gluconate as a carbon source at normal rates provided methylamine was supplied as a nitrogen source. Under these conditions high levels of transaldolase were observed.

Partial purification of the transaldolases synthesized by gluconate-grown cells of wild type *Arthrobacter* P1 and methylamine-grown cells of one of these mutants, strain Art 98, revealed the presence of transaldolase isoenzymes. These enzymes displayed similar kinetics but were very different in heat sensitivity. Functionally these isoenzymes are apparently very similar but their synthesis is regulated differently. One of the enzymes is synthesized constitutively whereas the other is specifically induced during growth on C₁ compounds. Strain Art 98 has lost the ability to synthesize the constitutive transaldolase. It is postulated that the C₁-induced transaldolase serves to ensure a sufficiently high rate of regeneration of ribulose-5-phosphate during growth on C₁ compounds.

Key words: *Arthrobacter* P1 — Transaldolase — Isoenzymes — Methylotrophy — RuMP cycle of formaldehyde fixation — Regulation — Gluconate — Methylamine — Xylose — Pentose phosphate pathway

The presence of the enzyme transaldolase (D-sedoheptulose-7-phosphate: D-glyceraldehyde-3-phosphate dihydroxyacetone transferase, EC 2.2.1.2) has been reported in a large variety of microorganisms, prokaryotes as well as eukaryotes. Its function is the interconversion, together with transketolase, of C₃ to C₇ sugar phosphates in the non-oxidative branch of the pentose phosphate pathway

(Tsolas and Horecker 1972). In these reactions the precursors for the biosynthesis of nucleic acids, ribose-5-phosphate, and aromatic amino acids, erythrose-4-phosphate, are formed (Fig. 1).

In addition to this anabolic function, transaldolase also plays a role in the metabolism of those carbon- and energy sources which are converted via the pentose phosphate pathway to fructose-6-phosphate and glyceraldehyde-3-phosphate. Examples of this are the metabolism of gluconate in a number of *Arthrobacter* species (Zagallo and Wang 1962; Levering et al. 1982) and the metabolism of glucose and xylose in the yeast *Candida utilis* (Tsolas and Horecker 1972).

A somewhat different function is fulfilled by transaldolase in autotrophic and methylotrophic microorganisms that employ either the Calvin cycle, the ribulose monophosphate (RuMP) cycle or the xylulose monophosphate cycle as the primary carbon assimilation pathway. In these organisms the enzyme may be involved in the regeneration of the C₁-acceptor molecule during autotrophic or methylotrophic growth (Quayle 1980; Anthony 1982). This has been demonstrated for instance in *Arthrobacter* P1, a facultative methylotroph able to grow rapidly on methylamine (Levering et al. 1981a). During growth of *Arthrobacter* P1 on C₁ compounds, formaldehyde is assimilated via the RuMP cycle which in this organism characteristically involves transaldolase and transketolase in the rearrangement reactions (Levering et al. 1982; Fig. 1).

During our studies on the regulation of C₁ metabolism in *Arthrobacter* P1 we tried to obtain mutant evidence for the suggested involvement of the so-called dissimilatory RuMP cycle of formaldehyde oxidation (Anthony 1982). This cycle involves glucose-6-phosphate and 6-phosphogluconate dehydrogenases as catalysts in energy-generating reactions during methylotrophic growth (Levering et al. 1981a; Fig. 1). As shown in Fig. 1 the metabolism of gluconate in *Arthrobacter* P1 is by way of the pentose phosphate pathway and, subsequently, the Embden-Meyerhof route (Levering et al. 1982). We therefore decided to try and isolate gluconate-negative mutants of *Arthrobacter* P1 deficient in 6-phosphogluconate dehydrogenase synthesis. Some of the gluconate-minus mutants that were obtained, however, were also unable to grow on xylose indicating that the metabolic blocks in these strains were in one of the enzymes of the non-oxidative branch of the pentose phosphate pathway. Contrary to our expectation, these mutants were still able to grow on methylamine. Subsequent studies revealed the presence of transaldolase isoenzymes in *Arthrobacter* P1.

Abbreviations. RuMP, ribulose monophosphate; DEAE, diethylaminoethyl

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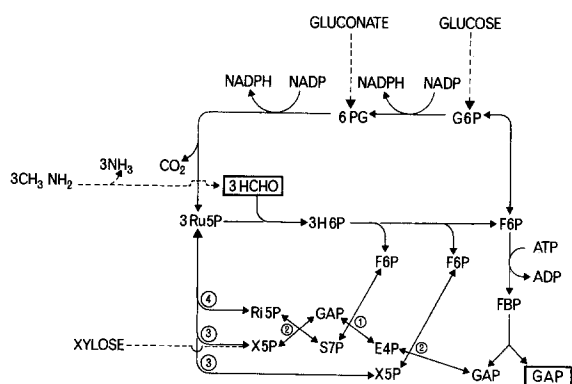


Fig. 1. Schematic representation of methylamine, glucose, gluconate and xylose metabolism in *Arthrobacter* P1. The various reactions involved in both the pentose phosphate pathway and the RuMP cycle, indicated by numbers, are catalyzed by the corresponding enzymes listed in Table 2. Abbreviations: *DHAP*, dihydroxyacetone phosphate; *GAP*, glyceraldehyde-3-phosphate; *E4P*, erythrose-4-phosphate; *Ru5P*, ribulose-5-phosphate; *Ri5P*, ribose-5-phosphate; *X5P*, xylulose-5-phosphate; *H6P*, hexulose-6-phosphate; *F6P*, fructose-6-phosphate; *FBP*, fructose-1,6-bisphosphate; *6PG*, 6-phosphogluconate; *G6P*, glucose-6-phosphate; *S7P*, sedoheptulose-7-phosphate

In this paper we report the result of studies on the regulation and function of transaldolase isoenzymes in wild type and mutant strains of *Arthrobacter* P1.

Materials and methods

Organisms

Arthrobacter P1 NCIB 11625 has been described previously (Levering et al. 1981a). Mutants of *Arthrobacter* P1 were isolated following UV light (254 nm) irradiation as described by Dijkhuizen et al. (1981). Among the survivors of this UV treatment strains were selected that had lost the ability to grow on gluconate. Both wild type and mutant strains were maintained on plates containing 1% (w/v) yeast extract and 1.2% (w/v) agar.

Medium and cultivation

Arthrobacter P1 wild type and the gluconate-negative strain Art 98 were grown at 30°C in 1-l conical flasks containing 250 ml of the mineral salts medium described by Levering et al. (1981a). Heat-sterilized solutions of carbon sources were added to the following final concentrations: glucose, 10 mM; potassium gluconate, 10 mM; sodium succinate, 15 mM; glycerol, 20 mM; and methylammonium chloride, 40 mM. Ammonia-free mineral salts medium was prepared as described by Levering and Dijkhuizen (1985). This medium was supplemented with methylammonium chloride (25 mM) as the nitrogen source and potassium gluconate as the carbon source. Incubation was on a rotary shaker at 200 rev./min.

Growth of the organism was monitored by measuring the absorbance of the cultures at 433 nm in a Vitatron 280 colorimeter (Vitatron, Dieren, The Netherlands). Cells for enzyme analysis were harvested, before they had reached the stationary growth phase by centrifugation at $6,000 \times g$ for 10 min at 4°C, washed once with 50 mM potassium

phosphate buffer pH 7.0 containing 5 mM $MgSO_4$ and resuspended in this buffer to a concentration of 5–10 mg dry weight/ml. These suspensions were used immediately for enzyme assays or stored at $-20^\circ C$ until required.

Substrate transition experiments

Cells for the substrate transition experiments were pregrown on methylamine as described above, harvested aseptically in the late-exponential growth phase by centrifugation at $6,000 \times g$ for 10 min at $20^\circ C$, washed once with mineral salts medium and resuspended in a small volume of this medium. These suspensions were subsequently used as inocula for 1-l fresh mineral salts medium, containing potassium gluconate (10 mM), in 3-l conical flasks. Incubation was on a rotary shaker at 250 rev./min and $30^\circ C$. Growth was monitored as described above and at appropriate time intervals samples were withdrawn from the cultures for the assay of enzyme activities. In the experiment with mutant strain Art 98 the culture was analysed for revertants by plating periodically on gluconate (10 mM) mineral salts-agar (1.2%, w/v).

Assays of enzyme activity

Cell-free extracts were prepared as described by Levering et al. (1981a). Spectrophotometric assays were performed with a Hitachi 100-60 spectrophotometer at $30^\circ C$. The following enzymes were assayed according to published procedures: 6-phosphogluconate dehydrogenase (NADP-dependent) (EC 1.1.1.44) according to van Dijken and Quayle (1977), except that 50 mM Tris-maleate pH 8.0 was used as a buffer; hexulose phosphate synthase, transaldolase (EC 2.2.1.2), transketolase (EC 2.2.1.1), ribulose-5-phosphate 3-epimerase (EC 5.1.3.1) and ribose-5-phosphate isomerase (EC 5.3.1.6) according to Levering et al. (1981a, 1982).

Purification of transaldolases

All steps were carried out at $0-4^\circ C$ unless stated otherwise. Both purification steps (ion exchange chromatography and gel permeation) were performed with a high-performance liquid chromatography (HPLC) system, which consisted of a Model 6000A solvent delivery system equipped with a U6K injector and a Model 441 absorbance detector (Waters Associates, The Netherlands). The purification procedure was as follows:

1. Preparation of crude extracts. *Arthrobacter* P1 and mutant strain Art 98 were grown on gluconate or methylamine, harvested by centrifugation, washed with 50 mM potassium phosphate buffer pH 7.0 and subsequently resuspended in a small volume of this buffer. These suspensions (3 ml; 50–75 mg dry wt/ml) were sonified with 1 g of glass beads at 20 kHz for 15×15 s with a MSE 100 W ultrasonic desintegrator (MSE Ltd., Crawley, Sussex, UK), with 45 s intermitting periods. Unbroken cells and debris were removed by centrifugation at $40,000 \times g$ for 10 min and the supernatant was again centrifuged at $40,000 \times g$ for 30 min. The pellet was discarded and the supernatant thus obtained contained approx. 25 mg protein/ml and was subjected to ion-exchange chromatography.

2. DEAE-polyol chromatography. Protein samples (1.0 ml) were injected in a column (9.5×500 mm) containing

diethylaminoethyl (DEAE)-Si 100 Polyol (Serva, Heidelberg, FRG) and eluted with a non-linear sodium chloride gradient (0–1 M) in 25 mM potassium phosphate buffer pH 7.0 at room temperature. The flow rate was set at 3.0 ml/min and 1.5 ml fractions were collected. Enzyme activities were measured as indicated above and peak fractions were pooled for further purification. The gradient was run with the aid of an Acorn Atom microcomputer (Acorn Computers Ltd., Cambridge, UK) interfaced with a three-way solenoid valve (type LFYA, The Lee Company, Westbrook, CT, USA) as described by van der Zee and Welling (1984).

3. Protein concentration. Before being subjected to gel filtration, the pooled fractions from the DEAE column were concentrated (25–30 \times) with a Minicon B-15 system (Amicon Co., Danvers, MA, USA).

4. Gel permeation chromatography. Samples (0.1 ml) containing 0.5–1.0 mg protein were applied to a Protein Pak 300-SW gel permeation column (7.5 \times 300 mm; Waters Associates, The Netherlands). Several runs were made with 0.2 M potassium phosphate buffer pH 7.0, with a flow rate of 0.5 ml/min at room temperature. Fractions (0.25 ml) were collected and analyzed for transaldolase activity. The fractions containing the highest activity were pooled and frozen at -20°C until required for further analysis.

Molecular weight determinations

Molecular weights of the purified transaldolase preparations were estimated with the gel filtration technique as described above, except that 0.05 ml samples containing 5–10 μg protein were injected. Chymotrypsinogen A, mw 25,000, albumin, mw 45,000 and albumin, mw 68,000 (5 μg of each; products of Boehringer, Mannheim, FRG) were used as marker proteins.

SDS-PAA gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis was performed according to Laemmli and Favre (1973) with the calibration proteins Combithek 104558 from Boehringer (Mannheim, FRG) as references. After electrophoresis the gels were stained with the silver staining technique as described by Wray et al. (1981).

Phosphate precipitation

Since high concentrations of phosphate inhibited transaldolase activity this compound was precipitated by adding a sufficient amount of calcium acetate to the enzyme preparations. The precipitate was removed by centrifugation and the supernatant thus obtained was subsequently used for kinetic studies. Calcium acetate up to 80 mM was shown not to inhibit the activity of transaldolase when added to assay mixtures for this enzyme.

Protein determination

Protein concentrations were determined according to Lowry et al. (1951), using bovine serum albumin as a standard.

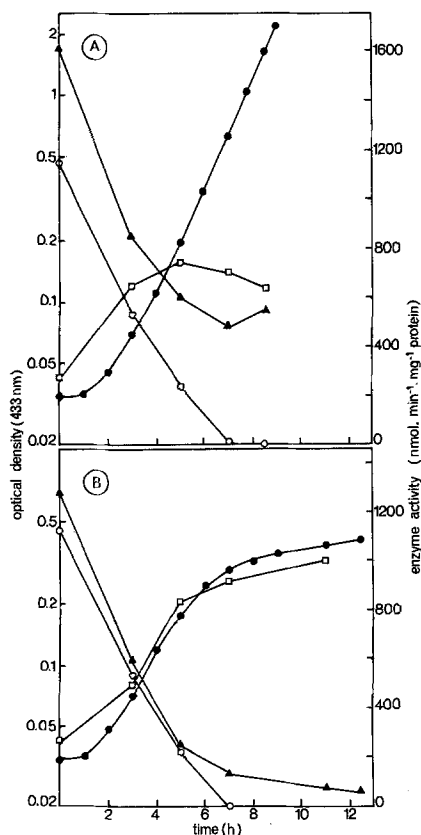


Fig. 2. A, B. Methyamine-gluconate substrate transition experiments with A wild type *Arthrobacter* P1 and B the gluconate-negative mutant Art 98. ●, Optical density; ○, hexulose phosphate synthase; ▲, transaldolase; □, 6-phosphogluconate dehydrogenase

Biochemicals

Erythrose-4-phosphate was obtained from Boehringer (Mannheim, FRG) as the diethylacetal monobarium salt. Transaldolase, purified from baker's yeast, was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other biochemicals were obtained from commercial sources and were of the highest available grade.

Results

Isolation and characterization of mutant strain Art 98

Following UV light irradiation five gluconate-negative strains of *Arthrobacter* P1 were isolated. A subsequent analysis of enzyme activities in cell-free extracts prepared from these mutants after growth on glucose indicated that 6-phosphogluconate dehydrogenase was present in normal activities. Thus, these mutants were either unable to take up gluconate from the medium, and/or phosphorylate it to 6-phosphogluconate, or were blocked in the non-oxidative branch of the pentose phosphate pathway (Fig. 1). Two of the gluconate-negative strains were also unable to utilize xylose as a carbon- and energy source and one of these mutants, designated strain Art 98, was subsequently studied in more detail.

Compared to wild type *Arthrobacter* P1, strain Art 98 exhibited significantly increased doubling times with glucose, glycerol or succinate (Table 1). From an analysis of the activities of the relevant enzymes (see above) in cell-free

Table 1

Doubling times of wild type *Arthrobacter* P1 and mutant strain Art 98 during growth on various substrates, and specific activities of transaldolase ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ of protein) in cell-free extracts

^a No growth

^b Enzyme activity not detectable

^c Data of Levering et al. (1982)

^d Enzyme activity present

nd Not determinable

Growth substrate	Doubling time (h)		Transaldolase activity	
	wild type	Art 98	wild type	Art 98
Gluconate	1.3	^a	508 ^c	nd
Xylose	1.4	^a	^d	nd
Glucose	1.2	2.2	437 ^c	^b
Glycerol	1.8	5.6	^d	^b
Succinate	1.3	2.0	^d	^b
Methylamine	2.5	2.5	1,397	1,218
Choline	2.2	2.6	238	60
Gluconate + methylamine minus $(\text{NH}_4)_2\text{SO}_4$	1.3	1.4	^d	1,286

extracts it became clear that the metabolic block in strain Art 98 is in transaldolase synthesis. After growth of strain Art 98 on these substrates no transaldolase activities were detectable, whereas wild type cells grown under the same conditions contained high levels of this enzyme (Table 1). Strain Art 98 did grow on methylamine with a doubling time of 2.5 h, as normally observed for wild type *Arthrobacter* P1. In cell-free extracts prepared from these cells high activities of transaldolase were detected and in case of strain Art 98 it could be shown, by plating out on gluconate agar, that this was not due to the appearance of revertants. Strain Art 98 also synthesized transaldolase during growth on choline, albeit in rather low levels. Further studies demonstrated that Art 98, although unable to grow on gluconate with ammonium sulphate as a nitrogen source, can use gluconate as a carbon- and energy source when incubated in the presence of methylamine as the sole nitrogen source. In the latter situation the growth was much faster than on methylamine alone and almost equalled the growth rates of wild type *Arthrobacter* P1 on gluconate/ammonia and gluconate/methylamine (Table 1). High activities of transaldolase were detected following growth on gluconate plus methylamine and again it could be shown that this was not caused by the presence of revertants.

The regulation of the synthesis of transaldolase in wild type *Arthrobacter* P1 and strain Art 98 was subsequently studied in substrate-transition experiments in which methylamine-grown cells were transferred into media containing gluconate as the sole carbon- and energy source and ammonia as the nitrogen source (Fig. 2). After incubation of *Arthrobacter* P1 in this medium growth started after 1 h and a doubling time of 1.3 h was reached in a few hours (Fig. 2A). An analysis of enzyme activities in cell-free extracts showed that hexulose phosphate synthase, an enzyme induced by formaldehyde during growth on methylamine (Levering et al. 1984, 1986a, b), decreased rapidly in activity and was no longer detectable after $t = 7$ h. The same response was observed for transaldolase, but the strong decrease in the activity of this enzyme levelled off, at $t = 6$ h, to reach a value of approximately $500 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein. The same activity is normally found in gluconate-grown cells of the organism (Table 1). Finally, the activity of 6-phosphogluconate dehydrogenase increased significantly upon transfer, reflecting its important role in the metabolism of gluconate in *Arthrobacter* P1 (Levering et al. 1982).

Upon incubation of methylamine-grown cells of mutant Art 98 in gluconate containing medium, a comparable

growth response was observed initially (Fig. 2B). From $t = 4-4.5$ h onwards, however, growth slowed down and, in accordance with the gluconate-minus phenotype of Art 98, almost came to a stop. This behaviour apparently is due to the disappearance of transaldolase activity in the culture. The level of this enzyme decreased rapidly during the first few hours following the transition and, unlike the situation with the wild type strain, almost reached zero levels (Fig. 2B). The hexulose phosphate synthase activities on the other hand followed almost exactly the same pattern as in the previous experiment, whereas 6-phosphogluconate dehydrogenase reached a somewhat higher level.

Two possible explanations were considered for the phenotype and characteristic properties of mutant Art 98. Assuming that only one transaldolase enzyme is present in *Arthrobacter* P1, the data indicate that in Art 98 this enzyme can only be induced by methylamine and choline, but not anymore by other substrates such as glucose, gluconate, glycerol, xylose and succinate. Alternatively, the data may be interpreted to indicate that *Arthrobacter* P1 is able to synthesize two transaldolase isoenzymes and that only one of these enzymes is expressed during growth on "heterotrophic" substrates, whereas the second is specifically induced by the C_1 compound methylamine, and by choline, the oxidation of which also results in the generation of formaldehyde (Levering et al. 1981 b). If this second assumption were correct it then follows that strain Art 98 must be blocked in the synthesis of the "heterotrophic" transaldolase. With the data available it was not possible to discriminate between these two possible explanations. We therefore decided to purify the transaldolases, from gluconate-grown cells of wild type *Arthrobacter* P1 and from methylamine-grown cells of strain Art 98, and to compare their properties.

Purification of transaldolases

The typical elution profiles obtained during DEAE-polyol chromatography of cell-free extracts of methylamine- or gluconate-grown cells of wild type *Arthrobacter* P1 and methylamine-grown cells of Art 98 are shown in Fig. 3A-C, respectively. Interestingly, the transaldolase activity of methylamine-grown cells of *Arthrobacter* P1 was separated in two distinct peaks of activity, whereas only one activity peak was observed in the two other preparations. The data in Fig. 3 further demonstrate that the major peak of activity found with methylamine-grown cells of wild type *Arthrobacter* P1 (Fig. 3A) was eluted from the column in

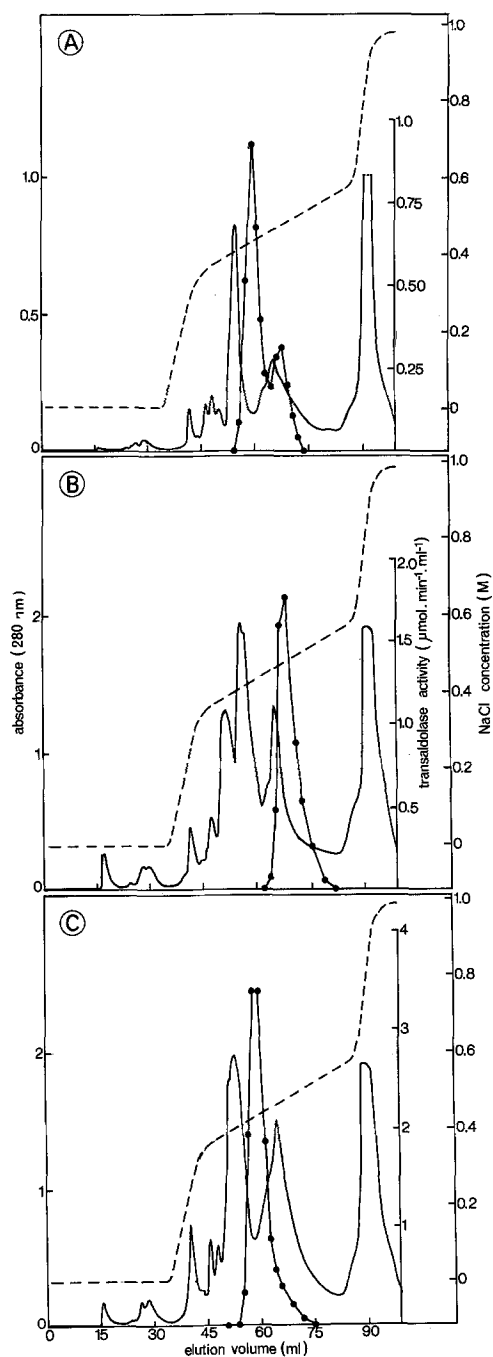


Fig. 3 A–C. DEAE-polyol chromatography elution profiles of cell-free extracts of **A** methylamine- or **B** gluconate-grown cells of wild type *Arthrobacter* P1 and **C** methylamine-grown cells of mutant Art 98. The transaldolase specific activities in these cell-free extracts were **A** 1,397, **B** 640, **C** 1,000 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ of protein. —, absorbance at 280 nm; — — —, sodium chloride gradient; ●, transaldolase

exactly the same fractions as the single peak found with methylamine-grown cells of mutant Art 98 (Fig. 3C). The same is true for the minor peak of activity in Fig. 3A and the single peak found with the gluconate-grown cells of wild type *Arthrobacter* P1 (Fig. 3B). Several runs with extracts from gluconate-grown cells of *Arthrobacter* P1 and methylamine-grown cells of Art 98 were performed and the pooled peak fractions obtained were combined, concentrated and subsequently subjected to gel permeation

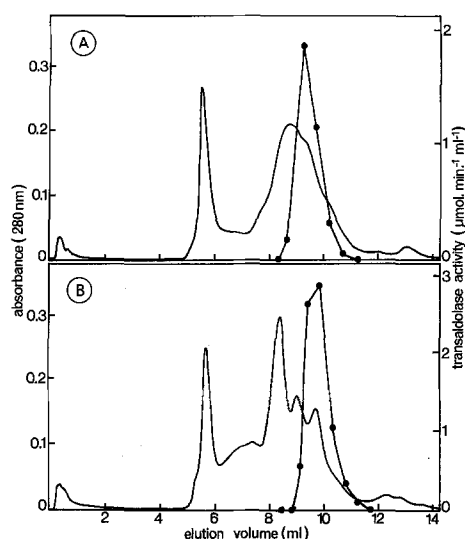


Fig. 4 A, B. Gel permeation chromatography elution profiles of the pooled peak fractions obtained during DEAE-polyol chromatography of cell-free extracts of **A** gluconate-grown cells of wild type *Arthrobacter* P1 and **B** methylamine-grown cells of Art 98. —, absorbance at 280 nm; ●, transaldolase

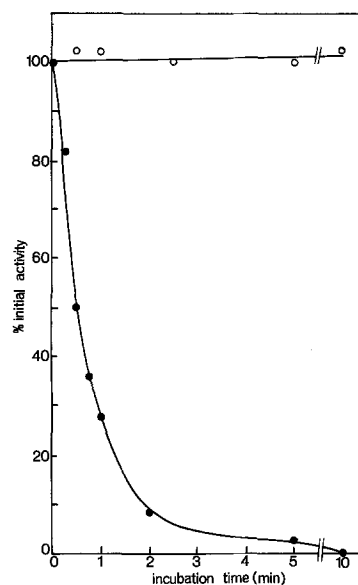


Fig. 5. Stability of transaldolase purified from gluconate-grown cells of *Arthrobacter* P1 (○) or methylamine-grown cells of Art 98 (●) during incubation at 50°C for various periods of time

chromatography. The typical elution profiles obtained with each of these preparations are shown in Fig. 4A and B, respectively. Again several runs were performed and the fractions containing the highest transaldolase activities were pooled and combined. The two preparations thus obtained were subsequently used for comparative studies of the two transaldolases.

Purity of the transaldolases. The specific activities of the transaldolase preparations obtained from gluconate-grown cells of *Arthrobacter* P1 and methylamine-grown cells of Art 98 were 16.0 and 27.5 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein, respectively. This means that overall a 25- and 27.5-fold purification, respectively, had been achieved (see Table 2).

Table 2

Relative activities of pentose phosphate pathway/RuMP cycle enzymes in cell-free extracts and preparations containing partially purified transaldolase of *Arthrobacter* P1 and mutant strain Art 98. The numbers refer to enzymes indicated in Fig. 1

A Cell-free extracts, transaldolase activities (100% values) in wild type and Art 98: 640 and 1,000 nmol · min⁻¹ · mg⁻¹ protein, respectively
 B Transaldolase preparations, idem: 16.0 and 27.5 µmol · min⁻¹ · mg⁻¹ protein, respectively

Enzyme	Percentage activity			
	<i>Arthrobacter</i> P1 gluconate-grown		strain Art 98 methylamine-grown	
	A	B	A	B
(1) Transaldolase	100	100	100	100
(2) Transketolase	68	0.7	172	0.1
(3) Ribulose-5-phosphate 3-epimerase	162	2.0	2,828	3.7
(4) Ribose-5-phosphate isomerase	63	4.7	200	2.2
Hexulose phosphate synthase	0	0	86	0

Activities of the three other enzymes operative in the pentose phosphate pathway/RuMP cycle in *Arthrobacter* P1, namely transketolase, ribose-5-phosphate isomerase and ribulose-5-phosphate 3-epimerase, were also detected in these transaldolase preparations. Compared to the transaldolase activities, however, the levels of these enzymes were rather low. This is especially the case when the relative activities of these enzymes in the cell-free extracts and the transaldolase preparations are considered (Table 2). It is therefore concluded that also with respect to these functionally closely related enzymes a considerable degree of purification of the transaldolases had been achieved.

Properties of the transaldolases

1. *pH optima.* Enzyme activities were determined over a range of pH values between 6.0 and 10.0. Both enzyme preparations reached maximum activities around pH 8.0, with very little change in values between pH 7.8 and 8.2. Similar observations have been reported for transaldolase purified from yeast (Venkataraman and Racker 1961a; Tsolas and Horecker 1972).

2. *Enzyme kinetics.* The apparent K_m values of the partially purified transaldolases for fructose-6-phosphate and erythrose-4-phosphate were estimated from Lineweaver-Burk plots. It was noticed, however, that the orthophosphate present in these preparations inhibited the activities of both transaldolases. This (reversible) inhibition became especially evident when, at submaximal concentrations of one of the substrates for transaldolase, larger amounts of the enzymes, and thus of phosphate, had to be added to the assay mixtures in order to obtain sufficiently high rates of product formation. The inhibitory effect of high phosphate concentrations on transaldolase activity is a well-known phenomenon and has for instance been reported for the yeast enzyme (Tsolas and Horecker 1972). Following the removal of phosphate (see Materials and methods) the apparent K_m values of the enzyme purified from strain Art 98 (methylamine-grown) for fructose-6-phosphate and erythrose-4-phosphate were estimated as 2.3 mM and 0.10 mM, respectively. Comparable values, 2.1 mM and 0.07 mM, respectively, were derived for the enzyme purified from wild type *Arthrobacter* P1 (gluconate-grown). When these kinetic studies were performed with transaldolase purified from Baker's yeast (Sigma Chemical Co., St. Louis, MO, USA) K_m values (1.2 mM and 0.08 mM, respectively) slightly higher than

reported in the literature (Venkataraman and Racker 1961a) were found.

3. *Molecular weights.* Using the HPLC gel permeation technique (see Materials and methods) molecular weights of 47,500 ($\pm 2,000$) and 52,000 ($\pm 2,000$) were determined for the enzymes purified from Art 98 and wild type, respectively. When using the same method, baker's yeast transaldolase appeared to have a molecular weight of about 64,000, which is in the same order of magnitude as reported for this enzyme by Venkataraman and Racker (1961b). When the transaldolase preparations of *Arthrobacter* P1 and Art 98 were subjected to SDS-polyacrylamide gel electrophoresis one major band in the 50,000 dalton region was observed in each case, indicating that both enzymes are monomeric in nature.

4. *Effects of temperature.* With both preparations transaldolase activities were found to increase with temperatures up to 40°C. When the temperature was further raised, maximal activities were observed in the range of 45–50°C with the enzyme isolated from the wild type, whereas the enzyme from Art 98 became very sensitive to inactivation. In the latter case no clear temperature optimum for the transaldolase reaction could be determined. The observed difference in temperature sensitivity was further investigated by incubating small amounts of the enzyme solutions, 20 µl containing approx. 3 µg of protein, in stoppered glass tubes at 50°C for various periods of time. After cooling on ice the remaining transaldolase activities in these samples were determined at 30°C. The results of this experiment (Fig. 5) indicated that, within 2 min of incubation at 50°C, the enzyme purified from Art 98 (methylamine-grown) lost 90% of its activity. No activity was detectable anymore after 10 min of incubation. In contrast, the enzyme isolated from *Arthrobacter* P1 (gluconate-grown) remained fully active when incubated at 50°C for prolonged periods of time. Interestingly, when a crude cell-free extract prepared from methylamine-grown cells of *Arthrobacter* P1 was subjected to this temperature treatment a rapid decrease in transaldolase activity, to approximately 40% of the initial level, was only observed in the first 2 min of incubation. No further loss of activity occurred upon prolonged incubation (data not shown).

Discussion

In this paper the isolation and characterization of a transaldolase-minus mutant of *Arthrobacter* P1, designated Art

98, is reported. Because of this mutation it has lost the ability to grow on gluconate and on xylose, substrates which are exclusively metabolized via the pentose phosphate pathway (Fig. 1; Levering et al. 1982). The observation that this mutant is still able to grow in mineral media with glucose, glycerol or succinate as carbon- and energy sources, albeit at lower rates compared to the wild type strain (Table 1), indicates that transaldolase is not really essential for growth of *Arthrobacter* P1 on these substrates. Under these conditions the intermediates of the pentose phosphate pathway required for biosynthetic reactions, i.e. ribose-5-phosphate and erythrose-4-phosphate, apparently are synthesized in strain Art 98 via 6-phosphogluconate/ribulose-5-phosphate and fructose-6-phosphate/glyceraldehyde-3-phosphate, respectively (Fig. 1). In contrast, mutants of *E. coli* lacking the functionally closely related enzyme transketolase are not only unable to grow on pentose sugars, but also in media with glucose or other carbon substrates unless supplemented with aromatic amino acids or a precursor, shikimic acid (Fraenkel and Vinopal 1973; Josephson and Fraenkel 1969, 1974). More complex but essentially similar observations have been made with transketolase-minus mutants of *Salmonella typhimurium* (Eidels and Osborn 1971) and *Bacillus subtilis* (Sasajima and Yoneda 1974; Sasajima and Kumada 1979, 1981).

During growth of *Arthrobacter* P1 on methylamine, the presence of transaldolase activity is considered essential for the regeneration of the formaldehyde-acceptor molecule ribulose-5-phosphate (Fig. 1; Levering et al. 1982). The observation that mutant Art 98, gluconate-negative because of a deficiency in transaldolase, was still able to grow on methylamine and *did* synthesize high levels of transaldolase under these conditions was rather puzzling at first (Table 1; Fig. 2). In order to investigate the possible synthesis of transaldolase isoenzymes in *Arthrobacter* P1 we decided to (partially) purify the enzymes present in gluconate-grown cells of *Arthrobacter* P1 and methylamine-grown cells of strain Art 98. Their subsequent characterization revealed that they have certain features in common, i.e. pH optima, kinetic parameters and inhibition by orthophosphate, but that they are clearly different in other respects. Slight but reproducible differences were found in their behaviour during the DEAE-polyol chromatography (Fig. 3B, C) and in the molecular weights of the enzymes (Fig. 4A, B). As was reported for two transaldolase isoenzymes in the yeast *Candida utilis* (Tsolas and Horecker 1970), the enzymes from *Arthrobacter* P1 and Art 98 showed a significant difference in sensitivity towards heat treatment. When incubated at 50°C the enzyme from Art 98 (methylamine-grown) rapidly lost its activity, whereas the enzyme from *Arthrobacter* P1 (gluconate-grown) remained stable (Fig. 5). In addition, the transaldolase activity present in methylamine-grown cells of *Arthrobacter* P1 wild type could be separated in two distinct peaks of activity during DEAE-polyol chromatography (Fig. 3A) and became only partially inactivated during incubation at 50°C. On the basis of these data it is concluded that wild type *Arthrobacter* P1 is able to synthesize two transaldolase isoenzymes. These data, and the observations made in the substrate transition experiments, also provide evidence for the conclusion that the enzyme purified from gluconate-grown cells of *Arthrobacter* P1 is synthesized constitutively whilst the other enzyme, purified from methylamine-grown cells of Art 98, is specifically induced in the presence of methylamine. It follows then that Art 98

has lost the ability to synthesize the transaldolase which normally becomes expressed constitutively.

Although the two transaldolases in *Arthrobacter* P1 are structurally apparently somewhat different, the available evidence indicates that they are functionally very similar, as is the case in *C. utilis* (Tsolas and Horecker 1972). This follows most clearly from the fact that, in the presence of methylamine as the sole nitrogen source, Art 98 is still able to utilize gluconate, reaching the same doubling time as normally observed with wild type *Arthrobacter* P1 (Table 1). The major difference between these two transaldolase enzymes therefore lies in the regulation of their synthesis. The additional recruitment via induction of a "methylotrophic" transaldolase during growth on C₁ compounds may simply serve to sufficiently enhance the flow of carbon via the rearrangement reactions in order to ensure continuous availability of the C₁-acceptor molecule ribulose-5-phosphate under these conditions. In view of the large differences in the activities of the enzymes of the non-oxidative pentose phosphate pathway/RuMP cycle (Tables 1 and 2; Levering et al. 1982) in methylamine- and gluconate-grown cells, it may well be that *Arthrobacter* P1 is able to additionally induce a complete set of these enzymes during growth on C₁ compounds.

The presence of isoenzymes among methylotrophic microorganisms is not a rare phenomenon (Bellion and Woodson 1975; Harder and Quayle 1971; Müller-Kraft and Babel 1983; O'Connor and Hanson 1975; this paper). As is the case in *Arthrobacter* P1, most of these "methylotrophic" isoenzymes clearly differ from the enzymes synthesized under "heterotrophic" growth conditions in the way they are regulated, either at the level of their synthesis or at the level of existing enzyme activity, or both. Since during methylotrophic and heterotrophic growth these enzymes fulfill different metabolic roles, the occurrence of these isoenzymes is clearly essential and apparently serves as a further mechanism of fine control of metabolic fluxes.

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References

- Anthony C (1982) The biochemistry of methylotrophs. Academic Press, London
- Bellion E, Woodson J (1975) Two distinct isocitrate lyases from a *Pseudomonas* species. J Bacteriol 122:557–564
- Dijken JP van, Quayle JR (1977) Fructose metabolism in four *Pseudomonas* species. Arch Microbiol 114:281–286
- Dijkhuizen L, Keijer, L, Waddell VJC, Primrose SB, Barstow D, Atkinson A (1981) The rapid isolation of mutants of some Gram-positive bacteria. FEMS Microbiol Lett 12:51–53
- Eidels L, Osborn MJ (1971) Lipopolysaccharide and aldoheptose biosynthesis in transketolase mutants of *Salmonella typhimurium*. Proc Natl Acad Sci USA 68:1673–1677
- Fraenkel DG, Vinopal RT (1973) Carbohydrate metabolism in bacteria. Annu Rev Microbiol 27:69–100
- Harder W, Quayle JR (1971) The biosynthesis of serine and glycine in *Pseudomonas* AM1 with special reference to growth on carbon sources other than C₁ compounds. Biochem J 121:753–762
- Josephson BL, Fraenkel DG (1969) Transketolase mutants of *Escherichia coli*. J Bacteriol 100:1289–1295

- Josephson BL, Fraenkel DG (1974) Sugar metabolism in transketolase mutants of *Escherichia coli*. J Bacteriol 118:1082–1089
- Laemmli UK, Favre K (1973) Maturation of the head of bacteriophage T4. I. DNA packing events. J Mol Biol 80:575–599
- Levering PR, Dijkhuizen L (1985) Regulation of methylotrophic and heterotrophic metabolism in *Arthrobacter* P1. Growth on mixtures of methylamine and acetate in batch and continuous cultures. Arch Microbiol 142:113–120
- Levering PR, Dijken JP van, Veenhuis M, Harder W (1981a) *Arthrobacter* P1, a fast growing versatile methylotroph with amine oxidase as a key enzyme in the metabolism of methylated amines. Arch Microbiol 129:72–80
- Levering PR, Binnema DJ, Dijken JP van, Harder W (1981b) Enzymatic evidence for a simultaneous operation of two one-carbon assimilation pathways during growth of *Arthrobacter* P1 on choline. FEMS Microbiol Lett 12:19–25
- Levering PR, Dijkhuizen L, Harder W (1982) Enzymatic evidence for the operation of the FBP aldolase cleavage and TK/TA rearrangement variant of the RuMP cycle in *Arthrobacter* P1. FEMS Microbiol Lett 14:257–261
- Levering PR, Dijkhuizen L, Harder W (1984) Metabolic regulation in the facultative methylotroph *Arthrobacter* P1. Growth on primary amines as carbon and energy sources. Arch Microbiol 139:188–195
- Levering PR, Croes LM, Tiesma L, Dijkhuizen L (1986a) Regulation of methylamine and formaldehyde metabolism in *Arthrobacter* P1. Effect of pulse-wise addition of “heterotrophic” substrates to C₁ substrate-limited continuous cultures. Arch Microbiol (in press)
- Levering PR, Croes LM, Dijkhuizen L (1986b) Regulation of methylamine and formaldehyde metabolism in *Arthrobacter* P1. Formaldehyde is the inducing signal for the synthesis of the RuMP cycle enzyme hexulose phosphate synthase. Arch Microbiol (in press)
- Lowry OH, Rosebrough NH, Farr AL, Randall RJ (1951) Protein measurements with the Folin phenol reagent. J Biol Chem 193:265–275
- Müller-Kraft G, Babel W (1983) Regulation der Citratsynthase bei fakultativ methylotrophen Bakterien. Z Allg Mikrobiol 23:181–187
- O'Connor ML, Hanson RS (1975) Serine transhydroxymethylase isoenzymes from a facultative methylotroph. J Bacteriol 124:985–996
- Quayle JR (1980) Microbial assimilation of C₁ compounds. Biochem Soc Transact 8:1–10
- Sasajima K, Kumada T (1979) Deficiency of D-glucose transport in transketolase mutant of *Bacillus subtilis*. Int Ferment Res Commun 9:17–26
- Sasajima K, Kumada T (1981) Change in the regulation of enzyme synthesis under catabolite repression in *Bacillus subtilis* pleiotrophic mutant lacking transketolase. Agric Biol Chem 45:2005–2012
- Sasajima K, Yoneda M (1974) D-Sedoheptulose-7-phosphate: D-glyceraldehyde-3-phosphate glycolaldehydetransferase and D-ribulose-5-phosphate 3-epimerase mutants of a *Bacillus* species. Agr Biol Chem 38:1305–1310
- Tsolas O, Horecker BL (1970) Isoenzymes of transaldolase in *Candida utilis*. Arch Biochem Biophys 136:287–319
- Tsolas O, Horecker BL (1972) Transaldolase. In: Boyer PD (ed) The enzymes, 3rd edn, vol 7. Academic Press, New York London, pp 259–280
- Venkataraman R, Racker E (1961 a) Mechanism of action of transaldolase. I. Crystallization and properties of yeast enzyme. J Biol Chem 236:1876–1882
- Venkataraman R, Racker E (1961 b) Mechanism of action of transaldolase. II. The substrate-enzyme intermediate. J Biol Chem 236:1883–1886
- Wray W, Boulikas T, Wray VP, Hancock R (1981) Silver staining of proteins in polyacrylamide gels. Anal Biochem 118:197–203
- Zagallo AC, Wang CH (1962) Comparative carbohydrate catabolism in *Arthrobacter*. J Gen Microbiol 29:389–401
- Zee R van der, Welling GW (1984) A personal computer-based gradient system for high-performance liquid chromatography with low-pressure mixing. J Chromatography 292:412–417

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